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(54) **PROCESS FOR PRODUCING OPTICALLY ACTIVE 3-PHENYL-1,3-PROPANEDIOL.**

(57) A process for producing optically active 3-phenyl-1,3-propanediol, which comprises treating an enantiomeric mixture of 3-phenyl-1,3-propanediol with an optionally treated microorganism and recovering an optically active enantiomer remaining intact. The microorganisms capable of leaving (R)-3-phenyl-1,3-propanediol intact include those belonging to the genera Candida, Hansenula, Rhodotorula, Protaminobacter, Aspergillus, Alternaria, Macrophomina, Preussia, and Talaromyces. The microorganisms capable of leaving (S)-3-phenyl-1,3-propanediol intact include those belonging to the genera Candida, Geotricum, Leucosporidium, Pichia, Torulaspora, Trichosporon, Escherichia, Micrococcus, Corynebacterium, Gordona, Rhodococcus, Aspergillus, Emericella, Absidia, Fusarium, Dactylium, Serratia and Pseudomonas.

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TECHNICAL FIELD

The present invention relates to a process for producing optically active 3-phenyl-1,3-propanediol. More particularly, the invention relates to a process for producing optically active 3-phenyl-1,3-propanediol characterized by permitting a microorganism or a preparation thereof to act on a mixture of enantiomers of 3-phenyl-1,3-propanediol and harvesting the residual optically active 3-phenyl-1,3-propanediol.

Optically active 3-phenyl-1,3-propanediol is an important intermediate for the synthesis of various medicinal compounds.

BACKGROUND ART

For the production of optically active 3-phenyl-1,3-propanediol, there is known a position-selective chemical reduction of 2,3-epoxycinnamyl alcohol [J. Org. Chem., 53(17), 4081 (1988)] as well as a chemical reduction of optically active 3-phenyl-3-hydroxypropionic acid [Tetrahedron Lett., 26(3), 351 (1985) and United States Patent No. 4921797].

However, the former process is not fully satisfactory in position selectivity and in terms of chemical purity. The latter process is also disadvantageous in that the optically active organic acid must be resolved with an optical resolution reagent beforehand and that the optical purity of the product optically active compound is low.

Under the circumstances, the establishment of an economical and expedient process for production of optically active 3-phenyl-1,3-propanediol of high optical purity has been demanded.

DISCLOSURE OF INVENTION

It is an object of the present invention to provide a process for producing optically active 3-phenyl-1,3-propanediol of high optical purity expediently and efficiently with the aid of a microorganism.

It is another object of the invention to provide a commercially useful process for producing optically active 3-phenyl-1,3-propanediol.

It is a further object of the invention to provide an efficient process for producing (R)-3-phenyl-1,3-propanediol or (S)-3-phenyl-1,3-propanediol with the aid of a microorganism.

The present inventors were interested in the utilization of a microorganism for the economical and expedient production of optically active 3-phenyl-1,3-propanediol of high optical purity and performed an extensive screening of microorganisms, mostly isolates from the soil, to find strains suited for the above purpose. As a consequence, they discovered that certain strains selected from certain genera and species of microorganisms act on a mixture of enantiomers of 3-phenyl-1,3-propanediol to leave either (R)-3-phenyl-1,3-propanediol or (S)-3-phenyl-1,3-propanediol. The present invention has been accomplished on the basis of the above finding.

The microorganisms to be employed in accordance with the invention may be any strain of microorganism that is able to act on a mixture of enantiomers of 3-phenyl-1,3-propanediol to leave either (R)-3-phenyl-1,3-propanediol or (S)-3-phenyl-1,3-propanediol.

The genera of those microorganisms which leave (R)-3-phenyl-1,3-propanediol include, among others, Candida, Hansenula, Rhodotorula, Protaminobacter, Aspergillus, Alternaria, Macrophomina, Preussia and Talaromyces.

The genera of those microorganisms which leave (S)-3-phenyl-1,3-propanediol include, among others, Candida, Geotrichum, Leucosporidium, Pichia, Torulaspora, Trichosporon, Escherichia, Micrococcus, Corynebacterium, Gordona, Rhodococcus, Aspergillus, Emericella, Absidia, Fusarium, Dactylium, Serratia and Pseudomonas.

Such a microorganism is generally grown in a culture medium and, then, submitted to the reaction with a mixture of enantiomers of 3-phenyl-1,3-propanediol. A preparation of such microorganism may instead be used in the reaction with a mixture of enantiomers of 3-phenyl-1,3-propanediol. Preferably the mixture of enatiomers is racemic 3-phenyl-1,3-propanediol.

DETAILED DESCRIPTION OF THE INVENTION

The microorganism to be used in accordance with the present invention may be any strain of microorganism that selectively utilizes one or the other enantiomer of 3-phenyl-1,3-propanediol and leaves the optically active (R) or (S) compound intact.

As such a microorganism, there may be employed any strain of microorganism that is able to act on a

mixture of enantiomers of 3-phenyl-1,3-propanediol to selectively leave (R)-3-phenyl-1,3-propanediol, said strain of microorganism being selected from the group of microorganisms belonging to the genus Candida, the genus Hansenula, the genus Rhodotorula, the genus Protaminobacter, the genus Aspergillus, the genus Alternaria, the genus Macrophomina, the genus Preussia and the genus Talaromyces; or any strain of microorganism that is able to act on a mixture of enantiomers of 3-phenyl-1,3-propanediol to selectively leave (S)-3-phenyl-1,3-propanediol, said strain of microorganism being selected from the group of microoganisms belonging to the genus Candida, the genus Geotrichum, the genus Leucosporidium, the genus Pichia, the genus Torulaspora, the genus Trichosporon, the genus Escherichia, the genus Micrococcus, the genus Corynebacterium, the genus Gordona, the genus Rhodococcus, the genus Aspergillus, the genus Emericella, the genus Absidia, the genus Fusarium, the genus Dactylium, the genus Serratia and the genus Pseudomonas.

As typical examples of the strain of microorganism that is able to act on a mixture of enantiomers of 3-phenyl-1,3-propanediol to leave (R)-3-phenyl-1,3-propanediol, there may be mentioned

the genus Candida: Candida lambica DSM 70090, etc.,

the genus Hansenula: Hansenula minuta DSM 70274, etc.,

the genus Rhodotorula: Rhodotorula rubra AHU 3945, AHU 3948, etc.,

the genus Protaminobacter: Protaminobacter ruber IAM 1081, etc.,

the genus Aspergillus: Aspergillus niger IFO 4414, Aspergillus ficuum IFO 4318, etc.,

the genus Alternaria: Alternaria kikuchiana IFO 5778, etc.,

the genus Macrophomina: Macrophomina phaseoli IFO 6696, etc.,

the genus Preussia: Preussia terricola IFO 7893, etc., and

the genus Talaromyces: Talaromyces flavus var. flavus IFO 7231, etc., and the like.

At least one strain of microorganism among them can be employed.

As typical examples of the strain of microorganism that is capable of acting on a mixture of enantiomers of 3-phenyl-1,3-propanediol to leave (S)-3-phenyl-1,3-propanediol, there may be mentioned

the genus Candida: Candida pintolopesii var. pintolopesii IFO 0729, etc.,

the genus Geotrichum: Geotrichum candidum IFO 4597, IFO 4598, IFO 5368, IFO 31810, JCM 1747, JCM 5222, Geotrichum fermentans JCM 2467, JCM 2468, Geotrichum klebahnii JCM 2171, etc.,

the genus Leucosporidium: Leucosporidium scottii IFO 1923, etc.,

the genus Pichia: Pichia quercuum DSM 70386, etc.,

the genus Torulaspora: Torulaspora delbrueckii IFO 0955, etc.,

the genus Trichosporon: Trichosporon capitatum IFO 1197, etc.,

the genus Escherichia: Escherichia coli IFO 3543, etc.,

the genus Micrococcus: Micrococcus luteus IFO 12708 etc.,

the genus Corynebacterium: Corynebacterium hoagii: JCM 1319, etc.,

the genus Gordona: Gordona rubro JCM 3199, etc.,

the genus Rhodococcus: Rhodococcus equi JCM 6820, Rhodococcus sp. JCM 6832, Rhodococcus maris JCM 6167, Rhodococcus fascians JCM 1316, Rhodococcus erythropolis IFO 12540, Rhodococcus rhodochrous DSM 43008, Rhodococcus coprophilus DSM 43302, Rhodococcus terrae DSM 43342, etc.,

the genus Aspergillus: Aspergillus niger IFO 4415, Aspergillus ficuum IFO 4320, etc.,

the genus Emericella: Emericella midulans IAM 2086, etc.,

the genus Absidia: Absidia coerulea JCM 5598, etc.,

the genus Fusarium: Fusarium solani IFO 5232, etc.,

the genus Dactylium: Dactylium dendroides ATCC 46032, etc.,

the genus Serratia: Serratia sp. No. 2664, Serratia sp. No. 2666, etc.,

the genus Pseudomonas: Pseudomonas Putida No. 2145B, etc., and the like.

At least one strain of microorganism among them can be employed.

The microorganisms identified hereinabove by IFO numbers are described in the "List of Cultures Ed. 8, Vol. 1 (1988)" published by Institute for Fermentation, Osaka (IFO), Japan and are available from the same Institute. The microorganisms designated by AHU numbers are listed in "Catalogue of Cultures Ed. 4 (1987)" published by Japan Federation of Culture Collections (JFCC) and are available from Faculty of Agriculture, Hokkaido University, Japan. The microorganisms designated by JCM numbers are listed in "Catalogs of Microbial Strains Ed. 4 (1989)" published by the Culture Collection of The Institute of Physical and Chemical Research, Japan and available from the same Culture Collection. The microorganisms designated by DSM numbers are listed in "Catalogs of Strains (1989)" of Deatsche Sammlung von Mikroorganismen (DSM) and are available from the same organization. The microorganisms designated by ATCC numbers have been deposited with American Type Culture Collection (ATCC) and are available from the same organization.

Serratia sp. No. 2664, Serratia sp. No. 2666 and Pseudomonas putida No. 2145B are novel strains which the present inventors have isolated from soil samples. All of these bacterial strains have been deposited with Fermentation Research Institute of the Agency of Industrial Science and Technology (communications to be addressed to Director of Fermentation Research Institute, the Agency of Industrial Science and Technology) as of May 22, 1991. The accession number of Serratia sp. No. 2664 is FERM P-12268, that of Serratia sp. No. 2666 is FERM P-12269 and that of Pseudomonas putida No. 2145B is FERM P-12270. The bacteriological characteristics and identifications of these strains are as follows.

Serratia sp. No. 2664

(a) Morphology	
(1) Cell shape and size	Rod
0.5-0.7 μm x 1.2-3.0 μm	
(2) Motility	Motile
(3) Sporulation	Non-sporulating
(4) Gram's stain	Negative
(b) Physiological characteristics	
(1) Oxidase	Negative
(2) Catalase	Positive
(3) Aminopeptidase	Positive
(4) Indole production	Negative
(5) VP test	Positive
(6) Denitrification	Positive
(7) Reduction of nitrate	Positive
(8) Citric acid utilization (Simons')	Positive
(9) Urease	Positive
(10) Phenylalanine deaminase	Negative
(11) Malonic acid utilization	Negative
(12) Levan formation from sucrose	Negative
(13) Lecithinase	Positive
(14) Starch hydrolysis	Negative
(15) Gelatin hydrolysis	Positive
(16) Casein hydrolysis	Positive
(17) DNA hydrolysis	Positive
(18) Tween 80 hydrolysis	Positive

(19) Esculin hydrolysis	Positive
(20) Lysis by 3 % KOH	Positive
(21) Aerobicity	Facultatively anaerobic
(22) Growth at 37°C	Positive
(23) Growth at 41°C	Positive
(24) Growth at pH 5.6	Positive
(25) Growth on Mac-Conkey Agar	Positive
(26) Growth on SS Agar	Positive
(27) Pigment production	Not produced
(28) OF test	F
(29) Gas production from glucose	-
(30) Acid production from sugars	
Glucose	+
Fructose	+
Xylose	+
Rhamnose	-
Sucrose	+
L-Arabinose	+
Melibiose	+
Trehalose	+
Lactose	-
Raffinose	-
Mannose	+
Maltose	+
Cellobiose	-
Melezitose	-

Adonitol	+
Inositol	+
Mannitol	+
Dulcitol	-
Sorbitol	+
Erythritol	-
Salicin	+
Glycerol	+
(31) ONPG (β-galactosidase)	Positive
(32) Arginine dihydrolase	Negative
(33) Lysine decarboxylase	Positive
(34) Ornithine decarboxylase	Positive
(35) Utilization of carbon sources	
Adipic acid	-
Caproic acid	+
Citric acid	+
Hippuric acid	-
Maleic acid	+
Phenylacetic acid	+
Glucose	+
Mannose	+
Maltose	+
Raffinose	-
Cellobiose	-
Adonitol	+
Mannitol	+
Nicotinic acid	-

Comparison of the above bacteriological characteristics with the relevant descriptions in Bergy's Manual of Systematic Bacteriology (1986) suggested that the above strain belongs to the genus Serratia. The strain was accordingly named Serratia sp. No. 2664.

Serratia sp. No. 2666

(a)	Morphology	
(1)	Cell shape and size	Rod
	0.5-0.7 µm x 1.2-3.0 µm	
(2)	Motility	Motile
(3)	Sporulation	Non-sporulating
(4)	Gram's stain	Negative
(b)	Physiological characteristics	
(1)	Oxidase	Negative
(2)	Catalase	Positive
(3)	Aminopeptidase	Positive
(4)	Indole production	Negative
(5)	VP test	Positive
(6)	Denitrification	Positive
(7)	Reduction of nitrate	Positive
(8)	Citric acid utilization (Simons')	Positive
(9)	Urease	Negative
(10)	Phenylalanine deaminase	Negative
(11)	Malonic acid utilization	Negative
(12)	Levan formation from sucrose	Negative
(13)	Lecithinase	Positive

(14) Starch hydrolysis	Negative
(15) Gelatin hydrolysis	Positive
(16) Casein hydrolysis	Positive
(17) DNA hydrolysis	Positive
(18) Tween 80 hydrolysis	Positive
(19) Esculin hydrolysis	Positive
(20) Lysis by 3 % KOH	Positive
(21) Aerobicity	Facultatively anaerobic
(22) Growth at 37°C	Positive
(23) Growth at 41°C	Positive
(24) Growth at pH 5.6	Positive
(25) Growth on Mac-Conkey Agar	Positive
(26) Growth on SS Agar	Positive
(27) Pigment production	Not produced
(28) OF test	F
(29) Gas production from glucose	-
(30) Acid production from sugars	
Glucose	+
Fructose	+
Xylose	+
Rhamnose	-
Sucrose	+
L-Arabinose	+
Melibiose	+
Trehalose	+
Lactose	-

Raffinose	-
Mannose	+
Maltose	+
Melezitose	-
Adonitol	+
Inositol	+
Mannitol	+
Dulcitol	-
Sorbitol	-
Erythritol	-
Salicin	+
Glycerol	+
(31) ONPG (β-galactosidase)	Positive
(32) Arginine dihydrolase	Negative
(33) Lysine decarboxylase	Positive
(34) Ornithine decarboxylase	Positive
(35) Utilization of carbon sources	
Adipic acid	-
Caproic acid	+
Citric acid	+
Hippuric acid	-
Maleic acid	+
Phenylacetic acid	+
Glucose	+
Mannose	+
Maltose	+
Raffinose	-

Cellobiose	-
Adonitol	+
Mannitol	+
Nicotinic acid	+

Comparison of the above bacteriological characteristics with the relevant descriptions in Bergy's Manual of Systematic Bacteriology (1986) suggested that the above strain belongs to the genus Serratia. The strain was accordingly named Serratia sp. No. 2666.

Pseudomonas Putida No. 2145B

(a)	Morphology	
(1)	Cell shape and size	0.5-0.8 μm x 1.5-3.0 μm
(2)	Motility	Motile
(3)	Sporulation	Non-sporulating
(4)	Gram's stain	Negative
(5)	Flagellum	Polar ($\geq$1)
(b)	Physiological characteristics	
(1)	Oxidase	Positive
(2)	Catalase	Positive
(3)	Aminopeptidase	Positive
(4)	Indole production	Negative
(5)	VP test	Negative
(6)	Denitrification	Negative
(7)	Reduction of nitrate	Negative
(8)	Urease	Negative

(9) Phenylalanine deaminase	Negative
(10) Levan formation from sucrose	Negative
(11) Lecithinase	Negative
(12) Starch hydrolysis	Negative
(13) Gelatin hydrolysis	Negative
(14) Casein hydrolysis	Negative
(15) DNA hydrolysis	Negative
(16) Tween 80 hydrolysis	Negative
(17) Esculin hydrolysis	Negative
(18) Tyrosine decomposition	Negative
(19) Lysis by 3 % KOH	Positive
(20) Aerobicity	Aerobic
(21) Growth at 37°C	Positive
(22) Growth at 41°C	Negative
(23) Growth at pH 5.6	Positive
(24) Growth on Mac-Conkey Agar	Positive
(25) Growth on SS Agar	Positive
(26) Growth on Cetrimid Agar	Positive
(27) Pigment Production	Produced
(28) OF test	O
(29) Gas production from glucose	-
(30) Acid production from sugars	
Glucose	+
Fructose	+
Xylose	+
(31) ONPG (β-galactosidase)	Negative
(32) Arginine dihydrolase	Positive

(33) Growth factor requirement	Negative
(34) Utilization of carbon sources	
Acetic acid	-
Adipic acid	-
Caproic acid	+
Citric acid	+
Citraconic acid	-
Glycolic acid	+
Levulinic acid	-
Maleic acid	+
Malonic acid	+
D/L mandelic acid	-
Phenylacetic acid	+
Sebacic acid	-
L-Tartaric acid	+
L-Arabinose	+
Trehalose	-
Fructose	+
Glucose	+
Mannose	+
Maltose	+
Xylose	-
Mannitol	+
Sorbitol	-
Gluconic acid	+
2-Ketogluconic acid	+
N-Acetylglucosamine	+

Glycine	-
D-Tryptophan	-
L-Tryptophan	-
Hippuric acid	-
Benzoyl formate	-
Benzylamine	+

Comparison of the above bacteriological characteristics with the relevant descriptions in Bergy's Manual of Systematic Bacteriology (1986) suggested that the above strain belongs to Pseudomonas putida. The strain was accordingly named Pseudomonas putida No. 2145B.

For the purposes of the invention, any of wild strains, mutants and recombinant strains which can be obtained by a genetic engineering technique such as cell fusion or gene manipulation, that is able to act on a mixture of enantiomers of 3-phenyl-1,3-propanediol to selectively leave an optically active compound can be advantageously employed.

A microorganism, such as the above, is usually grown in a culture medium and, then, submitted to the reaction with a mixture of enantiomers of 3-phenyl-1,3-propanediol.

The medium which is used for growing the strain for use in the invention is not critial in composition only if the selected strain may grow and multiply therein. The medium is generally a fluid medium containing sources of carbon and nitrogen and other nutrients. Any carbon source which the strain can utilize may be employed. As the sources of carbon, there may be employed various carbohydrates such as glucose, fructose, sucrose, dextrin, starch, etc., alcohols such as sorbitol, ethanol, glycerol, etc., organic acids such as fumaric acid, citric acid, acetic acid, propionic acid, etc. and the corresponding salts, hydrocarbons such as paraffin, and various mixtures thereof. The sources of nitrogen include, among others, inorganic acid ammonium salts such as ammonium chloride, ammonium sulfate, ammonium phosphate, etc., organic acid ammonium salts such as ammonium fumarate, ammonium citrate, etc., inorganic or organic nitrogenous materials such as meat extract, yeast extract, malt extract, peptone, corn steep liquor, casein hydrolysate, urea, etc., and various mixtures thereof. In the medium, there may be incorporated appropriate amounts of those nutrients which are commonly employed in the cultivation of microorganisms, such as inorganic salts, trace metal salts and vitamins. Where necessary, there may also be incorporated factors which may promote growth of the strain used and/or factors which may augment its ability to produce the object compound of the invention, such as a mixture of enantiomers of 3-phenyl-1,3-propanediol, as well as a buffer substance which may assist in the maintenance of the medium at a given pH.

The cultivation of the microorganism is carried out under conditions optimal for the growth of the particular strain, for example at a medium pH in the range of about 3.0 to 9.5, preferably about 4 to 8, and an incubation temperature in the range of about 20 to 45 °C, preferably about 25 to 37 °C. The cultivation may be aerobic or anaerobic. The cultivation time may, for example, be 5 to 120 hours, preferably about 12 to 72 hours.

The proportions of (R) and (S) in the substrate mixture of enantiomers of 3-phenyl-1,3-propanediol are not critical but it is advantageous for commercial purposes to employ a racemic form of 3-phenyl-1,3-propanediol.

The desired optically active 3-phenyl-1,3-propanediol is produced as a mixture of enantiomers of 3-phenyl-1,3-propanediol is added to a cell dispersion of the microorganism. The method of production of optically active 3-phenyl-1,3-propanediol from a mixture of enantiomers of 3-phenyl-1,3-propanediol may, for example, be whichever of the following alternatives: the method which comprises adding a mixture of enantiomers to a culture broth as such and the method which comprises separating the microbial cells from the culture broth, e.g. by centrifugation, resuspending the cells, either as they are or after washing with water, in a buffer solution, water or the like, and adding a mixture of enantiomers of 3-phenyl-1,3-propanediol to the resulting cell suspension. There are cases in which this reaction proceeds with advantage in the presence of a carbon source, such as glucose or sucrose, which serves as an energy source.

The optimal cell concentration of the reaction system cannot be stated in general terms, for it is

significantly dependent on the species or strain of microorganism employed. However, the concentration should be in the range where the efficiency of leaving the desired optically active compound intact will not be adversely affected. A typical cell concentration may for example be, on a dry cell basis, about 0.1 to 100 g/liter and preferably about 1 to 50 g/liter.

The cells may be wet viable cells or any preparation thereof, such as disrupted cells, acetone-treated cells, lyophilized cells and so on. These cells or cell preparations may be immobilized by known techniques such as the polyacrylamide gel method, sulfur-containing polysaccharide gel method (e.g. carrageenin gel method), alginic acid gel method, agar gel method and so on. The enzyme purified from such a cell preparation can also be employed. The enzyme can be obtained by using known purification processes in a suitable combination.

The mixture of enantiomers of 3-phenyl-1,3-propanediol can be used as it is or in the form of a solution in water or an organic solvent which will not interfere with the reaction or a dispersion prepared with a surfactant. The mixture of enantiomers of 3-phenyl-1,3-propanediol may be added in bolus at the beginning of the reaction or in several installments.

The reaction conditions can be selected from the ranges that will not detract from the yield of the object compound. For example, the pH of the reaction system can be selected from the range of pH about 3 to 10 and preferably pH about 5 to 9. The reaction temperature can be selected from the range of, for example, 10 to 60 °C and preferably from 20 to 40 °C. The reaction can be conducted with stirring or under stationary conditions for about 1 to 120 hours. As a tendency, the longer the reaction time, the smaller the residual amount of the desired 3-phenyl-1,3-propanediol compound but the higher is the optical purity of the 3-phenyl-1,3-propanediol. The concentration of a mixture of enantiomers of 3-phenyl-1,3-propanediol as the substrate is not particularly critical and is preferably about 0.1 to 20 weight % and more preferably about 0.2 to 10 weight %.

The optically active 3-phenyl-1,3-propanediol produced by the reaction, which remains in the reaction system, can be harvested by the separation and purification procedures generally known. For example, the optically active 3-phenyl-1,3-propanediol can be easily obtained by subjecting the reaction mixture, directly or after separation of the cells, to the conventional purification procedure such as extraction with an organic solvent, distillation and column chromatography. The optical purity of optically active 3-phenyl-1,3-propanediol can be measured by high performance liquid chromatography (HPLC) using an optical resolution column.

INDUSTRIAL APPLICABILITY

The optically active 3-phenyl-1,3-propanediol obtainable by the process of the invention is of value, for example as an important intermediate for the synthesis of tomoxethine which is an antidepressant agent.

The following examples are intended to illustrate the invention in further detail and should by no means be construed as delimiting the scope of the invention.

EXAMPLES

In the examples, the quantitative and optical purity determinations of 3-phenyl-1,3-propanediol in reaction mixtures were carried out by subjecting the optically active 3-phenyl-1,3-propanediol obtained by the reaction directly to high performance liquid chromatography using an optical resolution column (column: Chiralcel OB, Daicel Chemical Industries, Ltd.; solvent: n-hexane-isopropyl alcohol = 19:1; wavelength: 254 nm; flow rate: 1.0 ml/min.; column temperature: 40 °C; injection volume: 10 µl). Under the above operating conditions, the reaction time of 3-phenyl-1,3-propanediol was 13.1 minutes for (S) and 16.4 minutes for (R).

Example 1

A 500-ml Sakaguchi flask was charged with 50 ml of the following growth medium and, after sterilization, was inoculated with one of the microbial stains shown in Table 1. The inoculated flask was incubated under shaking at 30 °C for 48 hours.

Growth medium	
Glucose	2.0 weight %
Yeast extract	0.3 weight %
Peptone	0.5 weight %
Malt extract	0.3 weight %
pH	6.0

Then, 0.25 g of racemic 3-phenyl-1,3-propanediol was added to the culture broth containing the cells and the reaction was conducted on a reciprocating shaker at 30 °C for 96 hours.

After completion of the reaction, the cells were removed from the reaction mixture by centrifugation and the supernatant was extracted with 50 ml of ethyl acetate. The ethyl acetate extract was dehydrated over anhydrous sodium sulfate and the solvent was removed to give a syrup.

The syrup was dissolved in 50 ml of hexane-isopropyl alcohol (50/50) and the residual amount, absolute configuration and optical purity of the optically active 3-phenyl-1,3-propanediol were determined. The results are set forth in Table 1.

Table 1

Name of Microorganism	Absolute configuration	Optical purity (% e.e.)	Residual amount of 3-phenyl-1,3-propanediol (mg)
Candida pintolopesii var. pintolopesii IFO 0729	S	95	100
Geotrichum candidum IFO 4597	S	95	120
Geotrichum candidum IFO 4598	S	91	120
Geotrichum candidum IFO 5368	S	94	110
Geotrichum candidum IFO 31810	S	55	160
Geotrichum candidum JCM 1747	S	71	140
Geotrichum candidum JCM 5222	S	63	140
Geotrichum fermentans JCM 2467	S	56	150
Geotrichum fermentans JCM 2468	S	90	120

Table 1 (continued)

Name of Microorganism	Absolute configuration	Optical purity (% e.e.)	Residual amount of 3-phenyl-1,3-propanediol (mg)
Geotrichum kiebahnii JCM 2171	S	95	90
Leucosporidium scottii IFO 1923	S	43	110
Pichia quercuum DSM 70386	S	59	160
Torulaspora delbrueckii IFO 0955	S	74	120
Trichosporon capitatum IFO 1197	S	80	120
Candida lambica DSM 70090	R	95	100
Hansenula minuta DSM 70274	R	58	150
Rhodotorula rubra AHU 3945	R	81	120
Rhodotorula rubra AHU 3948	R	89	120

Example 2

A 500-ml Sakaguchi flask was charged with 50 ml of the following growth medium and, after sterilization, was inoculated with one of the strains mentioned in Table 2. The inoculated flask was incubated under shaking at 30°C for 48 hours.

Growth medium	
Meat extract	1.0 weight %
Peptone	1.0 weight %
Sodium chloride	0.5 weight %
pH	7.3

Then, 0.25 g of racemic 3-phenyl-1,3-propanediol was added to the culture broth containing the cells and the reaction was conducted on a reciprocating shaker at 30 °C for 48 hours. After completion of the reaction, the residual amount, absolute configuration and optical purity of 3-phenyl-1,3-propanediol were determined as in Example 1. The results are set forth in Table 2.

Table 2

Name of Microorganism	Absolute configuration	Optical purity (% e.e.)	Residual amount of 3-phenyl-1,3-propanediol (mg)
Protaminobacter ruber IAM 1081	R	74	100
Escherichia coli IFO 3543	S	90	90
Micrococcus luteus IFO 12708	S	48	100
Corynebacterium hoagii JCM 1319	S	94	70
Gordona rubro JCM 3199	S	100	80
Rhodococcus equi JCM 6820	S	100	90
Rhodococcus sp. JCM 6832	S	100	90
Rhodococcus maris JCM 6167	S	87	100
Rhodococcus fascians JCM 1316	S	100	80
Rhodococcus erythropolis IFO 12540	S	87	100
Rhodococcus rhodochrous DSM 43008	S	100	70
Rhodococcus coprophilus DSM 43302	S	74	100
Rhodococcus terrae DSM 43342	S	100	70

Example 3

The same procedures as in Example 1 were performed except that a 500-ml Sakaguchi flask was charged with 50 ml of the folloiwng growth medium and, after sterilization, was inoculated with one of the strains mentioned in Table 3. The residual amount, absolute configuration and optical purity of 3-phenyl-1,3-propanediol were determined as in Example 1. The results are set forth in Table 3.

Growth medium	
Potato (200 g) infusion	1000 ml
Glucose	20 g
Yeast extract	2 g
pH	5.6

Table 3

Name of Microorganism	Absolute configuration	Optical purity (% e.e.)	Residual amount of 3-phenyl-1,3-propanediol (mg/ml)
Aspergillus niger IFO 4414	R	64	5.0
Aspergillus ficuum IFO 4318	R	40	5.2
Alternaria kikuchiana IFO 5778	R	46	6.3
Macrophomina phaseoli IFO 6696	R	46	6.5
Preussia terricola IFO 7893	R	42	6.5
Talaromyces flavus var. flavus IFO 7231	R	89	4.6
Aspergillus niger IFO 4415	S	44	6.8
Aspergillus ficuum IFO 4320	S	82	4.4
Emericella midulans IAM 2086	S	60	5.2
Absidia coerulea JCM 5598	S	66	6.6
Fusarium solani IFO 5232	S	78	5.0
Dactylium dendroides ATCC 46032	S	50	6.8

Example 4

A 2-liter Sakaguchi flask was charged with 500 ml of a medium (pH 7) containing 1 % of glucose, 0.5 % of yeast extract, 0.3 % of polypeptone, 0.2 % of ammonium sulfate and 0.05 % of magnesium sulfate heptahydrate and heat-sterilized. This medium was inoculated with Serratia sp. No. 2664 and incubated on a reciprocal shaker at 30°C for 24 hours. The resulting broth was centrifuged to separate the cells, which were washed with physiological saline solution. The cells were resuspended in sufficient water to make 95 ml and the suspension was put in a 500-ml Sakaguchi flask. Then, 5 ml of a 20 % (w/v) aqueous solution of recemic 3-phenyl-1,3-propanediol was added and the mixture was incubated on a reciprocating shaker at 30°C for 72 hours. After completion of the reaction, the culture broth was centrifuged to separate the cells and the supernatant extracted twice with 100 ml portions of ethyl acetate. The organic layers wre pooled and dehydrated over anhydrous sodium sulfate and the solvent was evaporated off to give a syrup.

The syrup was dissolved in 10 ml of hexane-isopropyl alcohol (50:50) and the yield, absolute configuration and optically purify of the optically active 3-phenyl-1,3-propanediol were determined as in Example 1. The yield was found to be 37 %, the absolute configuration to be S, and the optical purity to be 96 % e.e.

Example 5

The cultivation, reaction and purification procedures of Example 4 were repeated except that Serratia sp. No. 2666 was used in lieu of Serratia sp. No. 2664 and the product was analyzed. The yield was found to be 40 %, the absolute configuration to be S, and the optical purity to be 98 % e.e.

Example 6

The cultivation, reaction and purification procedures of Example 4 were repeated except that Pseudomonas putida No. 2145B was used in lieu of Serratia sp. No. 2664 and the product was analyzed. The yield was found to be 38 %, the absolute configuration to be S, and the optical purity to be 90 % e.e.

Claims

1. A process for producing optically active 3-phenyl-1,3-propanediol which comprises permitting a microorganism or a preparation thereof to act on a mixture of enantiomers of 3-phenyl-1,3-propanediol and harvesting the residual optically active 3-phenyl-1,3-propanediol.

2. A process for producing optically active 3-phenyl-1,3-propanediol according to claim 1, wherein said microorganism is a strain of microorganism that is capable of acting on a mixture of enantiomers of 3-phenyl-1,3-propanediol to selectively leave (R)-3-phenyl-1,3-propanediol, said strain of microorganism being selected from the group of microorganisms belonging to the genus Candida, the genus

Hansenula, the genus Rhodotorula, the genus Protaminobacter, the genus Aspergillus, the genus Alternaria, the genus Macrophomina, the genus Preussia and the genus Talaromyces.

3. A process for producing optically active 3-phenyl-1,3-propanediol according to claim 2, wherein the strain of microorganism capable of leaving (R)-3-phenyl-1,3-propanediol is Candida lambica DSM 70090, Hansenula minuta DSM 70274, Rhodotorula rubra AHU 3945, AHU 3948, Protaminobacter ruber IAM 1081, Aspergillus niger IFO 4414, Aspergillus ficuum IFO 4318, Alternaria kikuchiana IFO 5778, Macrophomina phaseoli IFO 6696, Preussia terricola IFO 7893 or Talaromyces flavus var. flavus IFO 7231.

4. A process for producing optically active 3-phenyl-1,3-propanediol according to claim 1, wherein said microorganism is a strain of microorganism capable of acting on a mixture of enantiomers of 3-phenyl-1,3-propanediol to leave (S)-3-phenyl-1,3-propanediol, said strain of microorganism being selected from the group of microorganisms belonging to the genus Candida, the genus Geotrichum, the genus Leucosporidium, the genus Pichia, the genus Torulaspora, the genus Trichosporon, the genus Escherichia, the genus Micrococcus, the genus Corynebacterium, the genus Gordona, the genus Rhodococcus, the genus Aspergillus, the genus Emericella, the genus Absidia, the genus Fusarium, the genus Dactylium, the genus Serratia and the genus Pseudomonas.

5. A process for producing optically active 3-phenyl-1,3-propanediol according to claim 4, wherein said strain of microorganism capable of leaving (S)-3-phenyl-1,3-propanediol is Candida pintolopesii var. pintolopesii IFO 0729, Geotrichum candidum IFO 4597, IFO 4598, IFO 5368, IFO 31810, JCM 1747, JCM 5222, Geotrichum fermentans JCM 2467, JCM 2468, Geotrichum klebahnii JCM 2171, Leucosporidium scottii IFO 1923, Pichia quercuum DSM 70386, Torulaspora delbrueckii IFO 0955, Trichosporon capitatum IFO 1197, Escherichia coli IFO 3543, Micrococcus luteus IFO 12708, Corynebacterium hoagii JCM 1319, Gordona rubro JCM 3199, Rhodococcus equi JCM 6820, Rhodococcus sp. JCM 6832, Rhodococcus maris JCM 6167, Rhodococcus fascians JCM 1316, Rhodococcus erythropolis IFO 12540, Rhodococcus rhodochrous DSM 43008, Rhodococcus coprophilus DSM 43302, Rhodococcus terrae DSM 43342, Aspergillus niger IFO 4415, Aspergillus ficuum IFO 4320, Emericella midulans IAM 2086, Absidia coerulea JCM 5598, Fusarium solani IFO 5232, Dactylium dendroides ATCC 46032, Serratia sp. No. 2664 (FERM P-12268), Serratia sp. No. 2666 (FERM P-12269) or Pseudomonas putida No. 2145B (FERM P-12270).

6. A process for producing optically active 3-phenyl-1,3-propanediol according to claim 1, wherein said mixture of enantiomers of 3-phenyl-1,3-propanediol is racemic 3-phenyl-1,3-propanediol.

7. A process for producing optically active 3-phenyl-1,3-propanediol according to claim 1, which comprises growing a microorganism in a fluid medium and adding said mixture of enantiomers of 3-phenyl-1,3-propanediol to a cell dispersion.

8. A process for producing optically active 3-phenyl-1,3-propanediol according to claim 1, which comprises permitting said microorganism or preparation thereof to act on said mixture of enantiomers of 3-phenyl-1,3-propanediol at pH 3 to 10 and a temperature of 10 to 60 °C.

9. A process for producing optically active 3-phenyl-1,3-propanediol according to claim 1, wherein the concentration of said mixture of enantiomers of 3-phenyl-1,3-propanediol is 0.1 to 20 weight %.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP91/01064

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[5] C12P41/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C12P41/00, C12P7/22

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

COMPUTER SEARCH (CAS DATABASES)

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	US, A, 4921798 (01. 05. 90) & WO, A, 91/04334	1-9

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
October 22, 1991 (22. 10. 91)	November 11, 1991 (11. 11. 91)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	

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